

University of Groningen

Characterization of *Staphylococcus pseudintermedius* isolated from diseased dogs in Lithuania

Ruzauskas, M.; Couto, N.; Pavilonis, A.; Klimiene, I.; Siugzdiniene, R.; Virgailis, M.; Vaskeviciute, L.; Anskiene, L.; Pomba, C.

Published in:
Polish Journal of Veterinary Sciences

DOI:
[10.1515/pjvs-2016-0002](https://doi.org/10.1515/pjvs-2016-0002)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2016

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Ruzauskas, M., Couto, N., Pavilonis, A., Klimiene, I., Siugzdiniene, R., Virgailis, M., Vaskeviciute, L., Anskiene, L., & Pomba, C. (2016). Characterization of *Staphylococcus pseudintermedius* isolated from diseased dogs in Lithuania. *Polish Journal of Veterinary Sciences*, 19(1), 7-14. <https://doi.org/10.1515/pjvs-2016-0002>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

DOI 10.1515/pjvs-2016-0002

Original article

Characterization of *Staphylococcus pseudintermedius* isolated from diseased dogs in Lithuania

M. Ruzauskas¹, N. Couto², A. Pavilionis¹, I. Klimiene¹, R. Siugzdiniene¹,
M. Virgailis¹, L. Vaskeviciute¹, L. Anskiene³, C. Pomba²

¹ Institute of Microbiology and Virology, Lithuanian University of Health Sciences,
Tilzes g. 18, Kaunas, Lithuania

² Faculdade de Medicina Veterinaria, Universidade de Lisboa,
Avenida da Universidade Tecnica, 1300-477, Lisboa, Portugal

³ Department of Animal Reproduction, Lithuanian University of Health Sciences,
Tilzes g. 18, Kaunas, Lithuania

Abstract

The aim of this study was to characterize *Staphylococcus pseudintermedius* for its antimicrobial resistance and virulence factors with a special focus on methicillin-resistant (MRSP) strains isolated from sick dogs in Lithuania. Clinically sick adult dogs suffering from infections (n=214) and bitches with reproductive disorders (n=36) from kennels were selected for the study. Samples (n=192) from the 250 tested (76.8%) dogs were positive for *Staphylococcus* spp. Molecular profiling using the species-specific *nuc* gene identified 51 isolates as *S. pseudintermedius* (26.6% from a total number of isolated staphylococci) of which 15 isolates were identified as MRSP. Ten MRSP isolates were isolated from bitches with reproductive disorders from two large breeding kennels. Data on susceptibility of *S. pseudintermedius* to different antimicrobials revealed that all isolates were susceptible to vancomycin, daptomycin and linezolid. Two isolates (3.9%) were resistant to rifampicin. A high resistance was seen towards penicillin G (94.1%), tetracycline (64.7%) and macrolides (68.7%). Resistance to fluoroquinolones ranged from 25.5% (gatifloxacin) to 31.4% (ciprofloxacin). The most prevalent genes encoding resistance included *bla_Z*, *aac(6')-Ie-aph(2'')-Ia*, *mecA*, and *tet(M)*. The Luk-I gene encoding a leukotoxin was detected in 29% of the isolates, whereas the *siet* gene encoding exfoliative toxin was detected in 69% of the *S. pseudintermedius* isolates. This report of MRSP in companion animals represents a major challenge for veterinarians in terms of antibiotic therapy and is a concern for both animal and public health.

Key words: MRSP, companion animals, resistance, antimicrobials, toxins

Introduction

Staphylococcus pseudintermedius is an important opportunistic pathogen of companion animals, especially dogs (van Duijkeren et al. 2011). Canine infections caused by *S. pseudintermedius* are mostly skin infections, endometritis, cystitis and other less frequent infections (Cox et al. 1984, Morris et al. 2006). *S. pseudintermedius* has various virulence factors, including some that are closely related to the virulence factors of *S. aureus* (Futagawa-Saito et al. 2004b, Fitzgerald 2009). It produces enzymes such as coagulase, protease, thermonuclease and toxins, including haemolysins and exfoliative toxins (Fitzgerald 2009, Ben Zakour 2011). *S. pseudintermedius* also produces a leucotoxin known as Luk-I, which is very similar to Panton-Valentine leucocidin (PVL) from *S. aureus* (Futagawa-Saito et al. 2009, Futagawa Saito et al. 2004a).

Methicillin-resistant *S. pseudintermedius* (MRSP) has posed an increasing therapeutic challenge because of its limited treatment options. Colonization and infection caused by MRSP has been described in dogs, cats, horses, birds and humans. This fact demonstrates the zoonotical potential of *S. pseudintermedius*. It is also known that dogs can carry the same or similar MRSP strains for months without active infection. Several reports on isolates not susceptible to any antimicrobials authorized for use in veterinary medicine have been published causing veterinarians to consider using antimicrobials authorized for human medicine only. Good veterinary practice requires the use of antimicrobial treatment after correct diagnosis and susceptibility testing. However, reliable commercial identification systems for fast and correct identification of *S. pseudintermedius* are not currently available. In many cases, isolates will be erroneously identified as *S. intermedius* or *S. aureus*.

MRSP isolates are characterized by the presence of the *mecA* gene, which is located on staphylococcal cassette chromosome *mec* (SCC*mec*) elements and confers resistance to all β -lactam antibiotics. In addition to *mecA*, MRSP also may contain a wide range of antibiotic resistance genes. In addition to β -lactam resistance, resistance to 11 other antimicrobials was observed in a study of 103 epidemiologically unrelated MRSP isolates from dogs from Canada, the USA, Denmark, Germany, France, Italy, Sweden, Switzerland and the Netherlands. The resistance of *S. pseudintermedius* depends on geographical distribution as well as on other factors – thus, it is important to obtain data from different countries to better understand the epidemiological spread of resistance.

The aim of this study was to characterize *Staphylococcus pseudintermedius* in sick Lithuanian

dogs for antimicrobial resistance and virulence factors with a special focus on methicillin-resistant (MRSP) strains.

Materials and Methods

The investigations were carried out at the Lithuanian University of Health Sciences, Institute of Microbiology and Virology.

Sample collection

Two hundred and fifty dogs were randomly selected from small animal clinics located throughout the country as well as in breeding kennels of pure-breed dogs. Clinically sick adult dogs suffering from skin infections (n=155), otitis (n=48), respiratory tract infections (n=11) and bitches with reproductive disorders (metritis, temporal infertility, premature birth) (n=36) were selected for the study. The age ranged between 2 and 8 years. Anamnesis data showed that 190 dogs were untreated with antimicrobials at least 6 months before sampling; 45 sick dogs were treated over different intervals during the last 0-30 days before sampling. Fifteen bitches in kennels were prophylactically treated with fluoroquinolones and/or cephalosporins during the previous 3 months. Sterile cotton swabs with transport media (TRANS-WAB, Polysciences Inc.) or sterile syringes were used for collection of clinical samples. These samples were delivered to the laboratory on the same day. Only one sample from the affected organ was taken from each dog.

Bacteriological testing and DNA extraction

Samples were inoculated onto Mannitol-Salt Agar (Liofilchem, Italy) and Mannitol-Salt Agar supplemented with oxacillin (Sigma-Aldrich). Suspected colonies of *Staphylococcus* spp. were tested for presumptive genus identification according to the production of haemolysis, catalase, gram-staining, susceptibility to furazolidone, morphology and growing characteristics followed by a latex agglutination test („Staph Latex Kit”, Microgen, UK). Presumptive *S. pseudintermedius* isolates were identified up to species level using the RapID STAPH PLUS (Thermo Scientific) identification system and ERIC[®] manufacturer's software.

DNA material for molecular testing was obtained after bacterial lysis according to the extraction protocol prepared by the Community Reference Labora-

Table 1. Oligonucleotide primers used in this study.

Primer name	Sequence (5' – 3')	Size, bp and T (°C)	Target gene	Source
nuc1 nuc2	TRGGCAGTAGGATTCGTAA CTTTTGTGCTYCMTTTTGG	926 (58)	<i>nuc</i>	Sasaki et al. 2010
siet1 siet2	ATGGAAAATTTAGCGCATCTGG CCATTACTTTTCGCTTGTGTGC	359 (56)	exfoliative toxin	Lautz et al. 2006
lukS1 lukS2	TGTAAGCAGCAGAAAATGGGG GCCCCGATAGGACTTCTTACAA	503 (57)	<i>lukS</i>	Futagawa-Saito et al. 2004-1
lukF1 lukF2	CCTGTCTATGCCGCTAATCAA AGGTCATGGAAGCTATCTCGA	572 (57)	<i>lukF</i>	Futagawa-Saito et al. 2004-1
mecA1 mecA2	GGGATCATAGCGTCATTATTC AACGATTGTGACACGATAGCC	527 (61)	<i>mecA</i>	Poulsen et al. 2003
mecC1 mecC2	GCTCCTAATGCTAATGCA TAAGCAATAATGACTACC	204 (50)	<i>mecC</i>	Cuny et al. 2011
blaZ1 blaZ2	CAGTTCACATGCCAAAGAG TACACTCTTGCGGTTTC	772 (50)	<i>blaZ</i>	Schnellmann et al. 2006
tetM1 tetM2	GTAAATAGTGTCTTGGAG CTAAGATATGGCTCTAACAA	656 (45)	<i>tetM</i>	Aarestrup et al. 2000
tetK1 tetK2	TTAGGTGAAGGGTTAGGTCC GCAAACCTATTCCAGAAGCA	718 (55)	<i>tetK</i>	Aarestrup et al. 2000
aac6-aph2F aac6-aph2R	CAGAGCCTTGGGAAGATGAAG CCTCGTGTAATTCATGTTCTGGC	348 (61)	<i>aac(6')-Ie-aph(2'')-Ia</i>	Perreten et al. 2005
aph3-IIF aph3-IIR	CCGCTGCGTAAAAGATAC GTCATACCACTTGTCCGC	609 (57)	<i>aph(3')-IIIa</i>	Perreten et al. 2005
dfrK1 dfrK2	GCTGCGATGGATAAGAACAG GGACGATTTCACAACCATTAAGC	214 (50)	<i>dfrK</i>	Kadlec and Schwarz 2010
ermA1 ermA2	AAGCGGTAAAACCCCTCTGAG TCAAAGCCTGTCGGAATTGG	442 (53)	<i>ermA</i>	Jensen et al. 2002
ermC1 ermC2	ATCTTTGAAATCGGCTCAGG CAAACCCGTATTCCACGATT	295 (48)	<i>ermC</i>	Jensen et al. 2002
ermB1 ermB2	GGAACATCTGTGGTATGGCG CATTTAACGACGAAACTGGC	425 (48)	<i>ermB</i>	Jensen et al. 2002
msrA1 msrA2	GCTTAACATGGATGTGG GATTGTCTGTTAATTCCC	1230 (55)	<i>msrA</i>	Perreten et al. 2005
16S1 16S2	GTGCCAGCAGCCGCGGTAA AGACCCGGAACGTATTAC	886 (61)	<i>16S</i> staph	Poulsen et al. 2003

tory for Antimicrobial Resistance with slight modifications. Briefly, bacterial colonies were taken with a bacteriological loop from the surface of Mueller Hinton Agar and transferred to phosphate buffered saline (pH 7.3). The content was centrifuged for 5 min. The supernatant was then discarded and the pellet was re-suspended in Tris-EDTA (TE) buffer. The suspension was heated using a Biosan (Latvia) thermomixer to 100°C degrees for 10 minutes. The boiled suspension was transferred directly onto ice and diluted by 1:10 in TE.

Molecular testing

The species-specific thermonuclease (*nuc*) gene for *S. pseudintermedius* as well as the 16S rRNA gene was tested by PCR using oligonucleotides described previously (Poulsen et al. 2003, Sasaki et al. 2010). The positive control strain for *S. pseudintermedius* (previously confirmed by sequencing analysis of the 16S rRNA gene) was obtained from the Laboratory of Antimicrobial and Biocide Resistance, Technical University of Lisbon. Oligonucleotides used for detection

of antimicrobial resistance and virulence genes are presented in Table 1.

Antimicrobial susceptibility

Antimicrobial susceptibility testing was performed using the broth microdilution method. Sensitivity plates and the ARIS 2X automated system (Thermo Scientific) were used with the following antimicrobials: ceftriaxone, daptomycin, gatifloxacin, ciprofloxacin, clindamycin, erythromycin, gentamicin, levofloxacin, linezolid, oxacillin, penicillin, quinupristin/dalfopristin and rifampicin. Interpretation of results was carried out using the manufacturer's software (SWIN[®]) adapted to clinical breakpoints of the Clinical and Laboratory Standards Institute (CLSI). The quality control strain *S. aureus* ATCC 29213 was included in each assay for validation purposes.

PCR assays for antimicrobial and virulence genes

Detection of genes encoding antimicrobial resistance (*mecA*, *mecC*, *blaZ*, *tet(K)*, *tet(M)*, *erm(A)*, *erm(C)*, *msrA/B*, *aac(6')-Ie-aph(2'')-Ia*, *aph(3')-IIIa* and *dfxK*) was performed. Isolates were also tested for the *lukF/lukS* genes encoding leukocidin Luk-I and for the *siet* gene encoding exfoliative toxin. Annealing temperatures and oligonucleotides used are presented in Table 1. Positive control strains (previously confirmed by sequencing analysis) were obtained from the Laboratory of Antimicrobial and Biocide Resistance, Technical University of Lisbon.

Statistical analysis

Statistical analysis was performed using the „R 1.8.1” package (<http://www.r-project.org/>). Comparison between categorical variables was calculated using the chi-square test and Fisher's exact test. Results were considered statistically significant if $p < 0.05$.

Results

One hundred and ninety two samples from 250 tested (76.8%) were positive for the presence of *Staphylococcus* species. The percentage was higher in non-treated animals (89.5%). Fifty-four isolates did not ferment mannitol on mannitol-salt agar, had a positive reaction with Microgen Staph Latex Kit and expressed a large double zone of haemolysis on

sheep-blood agar. Thirty-two isolates were initially identified as *S. intermedius* using a biochemical identification system. Molecular typing using the species-specific *nuc* gene identified 51 isolates as *S. pseudintermedius* (26.6%) including those previously identified as *S. intermedius*. Fifteen samples (29.4%) from different dogs were able to grow on mannitol salt agar supplemented with 2 mg/L oxacillin. The *mecA* gene was detected in all of these isolates, all of them being resistant to oxacillin and identified as MRSP. The *mecC* gene was not detected. Ten MRSP isolates were isolated in two kennels (previously treated with fluoroquinolones and/or cephalosporins) breeding Yorkshire terriers, French Bulldogs and English Bulldogs (number of adult dogs in each kennel was 18 and 22 respectively).

Table 2 presents data on the distribution of *S. pseudintermedius* including MRSP isolates in dogs with different clinical infections, together with the number of isolates harbouring genes encoding production of Luk-I and *siet*.

The data presented in Table 2 demonstrate that *S. pseudintermedius* was isolated from dogs with different clinical infections, although the highest frequency of this species was detected in dogs with pyoderma. Ten MRSP isolates were isolated in two large breeding kennels from bitches with reproductive disorders.

Genes encoding production of Luk-I toxin were not associated with any of the clinical infections ($p > 0.5$); however, the *siet* gene, responsible for the production of an exfoliative toxin, was significantly associated with isolates from skin infections ($p < 0.01$). From all isolates, 68.6% had at least one gene responsible for the production of toxins.

Data on antimicrobial susceptibility of the isolates is presented in Table 3.

All *S. pseudintermedius* isolated strains were susceptible to vancomycin, daptomycin and linezolid. Two isolates (3.9%) were resistant to rifampicin. More frequent resistance was demonstrated to penicillin (94.1%), tetracycline (64.7%) and macrolides (68.7%). Resistance to fluoroquinolones was high and ranged from 25.5% (gatifloxacin) to 31.4% (ciprofloxacin). Genes encoding resistance to separate classes of antimicrobials were found in different numbers (Table 3). The most prevalent genes included *blaZ*, *aac(6')-Ie-aph(2'')-Ia*, *mecA*, and *tet(M)*.

Discussion

Bacteria of the genus *Staphylococcus* are highly prevalent in clinical samples from small animals. In general, we found that 76.8% of the tested samples were positive although the prevalence was higher in

Table 2. Clinical infections associated with *S. pseudintermedius* including MRSP and presence of the genes encoding toxins.

Clinical disorder	<i>S. pseudintermedius</i> isolates	Number of MRSP isolates	Genes encoding toxins		
			<i>lukS</i>	<i>lukF</i>	<i>siet</i>
Otitis	5	0	2	2	3
Pyoderma	24	4	6	6	22
Reproductive disorders	18	10	6	6	9
Other	4	1	1	1	1

Table 3. Antimicrobial susceptibility data and genes encoding resistance in *S. pseudintermedius* isolates (n=51).

Class of antimicrobials	Antimicrobial	Susceptibility ¹ , n (%)			Genes encoding resistance
		S	I	R	
Penicillins	Penicillin	3(5.9)	–	48(94.1)	<i>blaZ</i> (45) ³ <i>mecA</i> (15)
	Oxacillin	36(70.6)	–	15(29.4)	
Cephalosporins	Ceftriaxone	35(68.6)	3(5.9)	13(25.5)	
Tetracyclines	Tetracycline	18(35.3)	–	33(64.7)	<i>tet(K)</i> (6) <i>tet(M)</i> (14)
Macrolides, lincosamides and streptogramins	Erythromycin	14(27.5)	1(2.0)	36(70.6)	<i>erm(A)</i> (1)
	Clindamycin	14(27.5)	–	37(72.5)	<i>erm(C)</i> (3)
	Quinupristin/dalfopristin	48(94.1)	2(3.9)	1(2.0)	<i>msrA/B</i> (6)
DFR inhibitors	Trimethoprim	36(70.6)	–	15(29.4)	<i>dfrK</i> (8)
	Ciprofloxacin	35(68.7)	–	16(31.4)	nt
Fluoroquinolones	Gatifloxacin	38(74.5)	–	13(25.5)	nt
	Levofloxacin	36(70.6)	1(2.0)	14(27.5)	nt
Rifamycins	Rifampicin	49(96.1)	–	2(3.9)	nt
Lipopeptides	Daptomycin	51(100)	–	0(0)	nt
Glycopeptides	Vancomycin	51(100)	–	0(0)	nt
Aminoglycosides	Gentamicin	25(49.0)	7(13.7)	19(37.6)	<i>aac(6')-Ie-aph(2'')-Ia</i> (16) <i>aph(3')-IIIa</i> (11)
Oxazolidinones	Linezolid	51(100)	–	0(0)	nt

¹ S – susceptible; I – intermediate susceptible; R – resistant² nt – not tested³ in parentheses – number of isolates harbouring the tested genes

non-treated animals (89.5%). Such data are consistent with data obtained by other authors (Griffeth 2008, Penna et al. 2010). We focused on *S. pseudintermedius* since it is the most prevalent species in dogs (Hauschild and Wójcik 2007, Penna et al. 2010, Bannoehr and Guardabassi 2012). Moreover, *S. pseudintermedius* is often reported as methicillin-resistant with co-resistance to different classes of antimicrobials other than beta-lactams (Perreten et al. 2010, Weese and Duijkeren 2010, Windahl et al. 2012). The number of *S. pseudintermedius* isolates (26.6%) revealed that this species is widely distributed in clinical samples from dogs although there are other species that are prevalent as well (data not presented).

There are different data about the prevalence of *S. pseudintermedius* described by other authors. For

example, Feng et al. (2012) reported a prevalence of 18.3%, while Garbacz et al. (2013) described it to be 51.8.4% of tested animals. Data on the prevalence might depend on study design, identification methods, sampling, animal health status and other factors. It is known that classical biochemical tests for species identification are not always capable of identifying *S. pseudintermedius* (van Duijkeren et al. 2011). We found this to be true as well. Certain substrates (carbohydrates and amino acids) are weakly fermented and thus interpretation of results based on a colour index is subjective.

In our study *S. pseudintermedius* was isolated from dogs with different clinical infections, and thus we detected genes encoding for pathogenicity factors. We detected a high frequency (68.8%) of *luk* and/or *siet*

genes in *S. pseudintermedius* isolates in Lithuania. Interestingly, all isolates that harboured both *lukF* and *lukS* genes harboured the *siet* gene as well ($p < 0.01$) but not *vice versa*. To our knowledge such a statistical relationship had not been detected before. Statistically reliable results were obtained when studying the association between the presence of *siet* gene in isolates from skin infections compared with isolates obtained from other types of infection. This agrees with the fact that the exfoliative toxin is a virulence factor of *S. pseudintermedius* involved in canine pyoderma. It is mainly found among isolates from skin infections (Lautz et al. 2006, Iyori et al. 2010). The *luk* genes were found in different *S. pseudintermedius* strains irrespective of the source of isolation. The Luk-I shows strong leucotoxicity towards various polymorphonuclear cells (Futagawa et al. 2004a). It might be responsible for invasion of the host by suppressing its cellular immunity and could be produced by different strains of *S. pseudintermedius*.

Data on antimicrobial susceptibility revealed that *S. pseudintermedius* most frequently has resistance to antimicrobials used in dogs including penicillins (resistance attributed to *blaZ* gene), tetracyclines (*tet*(K) and *tet*(M) genes) and macrolides (*ermA* and *ermC* genes).

The *ermB* gene was not detected. In fact, this gene is rarely isolated (1.9%) in MRSP isolates from Europe and North America collected previously (Perreten et al. 2010), except in Norway where resistance of *S. pseudintermedius* was attributed to the *ermB* gene (Norstrom et al. 2009). A high rate of resistance to fluoroquinolones (25.5–31.4%) was also recorded. Resistance mechanisms to fluoroquinolones of *S. pseudintermedius* are well described (Descloux et al. 2008). The high frequency of resistance to fluoroquinolones found here could be explained in that fluoroquinolones are frequently used to treat dog infections especially in cases with unsatisfactory clinical practice where broad-spectrum antimicrobials are selected for treatment without sending clinical material to a laboratory for diagnosis and antibiogram. Resistance of *S. pseudintermedius* isolates to gentamicin was also high (37.6%). The genes responsible for encoding resistance to aminoglycosides *aac*(6')-Ie-aph(2'')-Ia and *aph*(3')-IIIa were detected here. The same genes were recently found in most isolates of enterococci isolated from diseased cows, pigs and poultry in Lithuania (Seputiene et al. 2012). Those genes encoding resistance to aminoglycosides were also found in *S. pseudintermedius* in other countries (Kadlec et al. 2010). It is interesting that resistant isolates to gentamicin harboured the *siet* gene as a rule ($p < 0.01$).

In this study, 29.4% of *S. pseudintermedius* strains were identified as MRSP. Lithuania-specific data on methicillin resistant staphylococci isolated from animals is scarce – the first case of methicillin resistance in livestock was only reported in 2011, when MRSA ST398 strains were found and characterised in pigs (Ruzauskas et al. 2013). To the best of our knowledge, this study is the first Lithuanian study where MRSP strains were confirmed using molecular methods. This high frequency of MRSP in dogs could be explained as follows: first, only diseased animals were involved in the study and most of them were being treated or had been treated previously with an antimicrobial. Second, as proven before, the use of fluoroquinolones and cephalosporins might select for antimicrobial resistant bacteria (SAGAM 2009, van Duijkeren et al. 2011) and some of these dogs had been previously treated with some of these antimicrobial drugs. Finally, 10 MRSP isolates were isolated on two large breeding kennels from bitches with reproductive disorders in which the owners irregularly used antimicrobials, including enrofloxacin and/or cefovecin for “better reproductive performance”. Such inappropriate use of antimicrobials possibly led to high resistance rates of MRSP in these kennels. Thus, breeding kennels might be a reservoir of MRSP strains and may pose a risk for spreading such strains during mating. There is no requirement for reporting MRSP or MRSA strains prevalent in kennels. Thus, other breeders have no information about the status of such animals. Attention should be paid to this problem since methicillin-resistant staphylococci pose a risk not only to animals but also to humans (Catry et al. 2010, Stegmann et al. 2010, van Duijkeren et al. 2011).

Our findings indicate that staphylococci including *S. pseudintermedius* are very common in clinical samples from diseased dogs. The prevalence of genes encoding toxins in *S. pseudintermedius* is high as also the resistance rates to some critically important antimicrobials such as beta-lactams and fluoroquinolones. Isolates remain susceptible only to those antimicrobials that are still banned from veterinary use (linezolid, vancomycin, and daptomycin). Such antibiotics should be reserved for humans and control of their use in kennels needs to be improved. Monitoring of antimicrobial resistance in kennels should be performed routinely and should include control options to avoid the spread of resistance.

Acknowledgements

This research was funded by grants (MIP-075/2013 and SIT-6/2015) from the Research Council of Lithuania.

References

- Aarestrup FM, Agerso Y, Gerner-Smidt P, Madsen M, Jensen LB (2000) Comparison of antimicrobial resistance phenotypes and resistance genes in *Enterococcus faecalis* and *Enterococcus faecium* from humans in the community, broilers, and pigs in Denmark. *Diagn Microbiol Infect Dis* 37: 127-137.
- Bannoehr J, Guardabassi L (2012) *Staphylococcus pseudintermedius* in the dog: taxonomy, diagnostics, ecology, epidemiology and pathogenicity. *Vet Dermatol* 23: 253-266.
- Ben Zakour NL, Bannoehr J, van den Broek AH, Thoday KL, Fitzgerald JR (2011) Complete genome sequence of the canine pathogen *Staphylococcus pseudintermedius*. *J Bacteriol* 193: 2363-2364.
- Catry B, van Duijkeren E, Pomba MC, Greco C, Moreno MA, Pyörälä S, Ruzauskas M, Sanders P, Threlfall EJ, Ungemach F, Törneke K, Munoz-Madeiro C, Torren-Edo J (2010) Reflection paper on MRSA in food producing and companion animals: epidemiology and control options for human and animal health. *Epidemiol Infect* 138: 626-644.
- Couto N, Pomba C, Moodley A, Guardabassi L (2011) Prevalence of methicillin-resistant staphylococci among dogs and cats at a veterinary teaching hospital in Portugal. *Vet Rec* 169: 72.
- Cox HU, Newman SS, Roy AF, Hoskins JD (1984) Species of *Staphylococcus* isolated from animal infections. *Cornell Vet* 74: 124-135.
- Cuny C, Layer F, Strommenger B, Witte W (2011) Rare occurrence of methicillin-resistant *Staphylococcus aureus* CC130 with a novel *mecA* homologue in humans in Germany. *PloS One* 6: e24360.
- Descloux S, Rossano A, Perreten V (2008) Characterization of new staphylococcal cassette chromosome *mec* (SCCmec) and topoisomerase genes in fluoroquinolone- and methicillin-resistant *Staphylococcus pseudintermedius*. *J Clin Microbiol* 46: 1818-1823.
- Feng Y, Tian W, Lin D, Luo Q, Zhou Y, Yang T, Deng Y, Liu YH, Liu JH (2012) Prevalence and characterization of methicillin-resistant *Staphylococcus pseudintermedius* in pets from South China. *Vet Microbiol* 160: 517-524.
- Fitzgerald JR (2009) The *Staphylococcus intermedius* group of bacterial pathogens: species re-classification, pathogenesis and the emergence of methicillin resistance. *Vet Dermatol* 20: 490-495.
- Futagawa-Saito K, Makino S, Sunaga F, Kato Y, Sakurai-Komada N, Ba-Thein W, Fukuyasu T (2009) Identification of first exfoliative toxin in *Staphylococcus pseudintermedius*. *FEMS Microbiol Lett* 301: 176-180.
- Futagawa-Saito K, Sugiyama T, Karube S, Sakurai N, Ba-Thein W, Fukuyasu T (2004a) Prevalence and characterization of leukotoxin-producing *Staphylococcus intermedius* in isolates from dogs and pigeons. *J Clin Microbiol* 42: 5324-5326.
- Futagawa-Saito K, Suzuki M, Ohsawa M, Ohshima S, Sakurai N, Ba-Thein W, Fukuyasu T (2004b) Identification and prevalence of an enterotoxin-related gene, *se-int*, in *Staphylococcus intermedius* isolates from dogs and pigeons. *J Appl Microbiol* 96: 1361-1366.
- Garbacz K, Ćarnowska S, Piechowicz L, Haras K (2013) Pathogenicity potential of *Staphylococcus pseudintermedius* strains isolated from canine carriers and from dogs with infection signs. *Virulence* 4: 255-259.
- Griffeth GC, Morris DO, Abraham JL, Shofer FS, Rankin SC (2008) Screening for skin carriage of methicillin-resistant coagulase-positive staphylococci and *Staphylococcus schleiferi* in dogs with healthy and inflamed skin. *Vet Dermatol* 19: 142-149.
- Hauschild T, Wójcik A (2007) Species distribution and properties of staphylococci from canine dermatitis. *Res Vet Sci* 82: 1-6.
- Iyori K, Hisatsune J, Kawakami T, Shibata S, Murayama N, Ide K, Nagata M, Fukata T, Iwasaki T, Oshima K, Hattori M, Sugai M, Nishifuji K (2010) Identification of a novel *Staphylococcus pseudintermedius* exfoliative toxin gene and its prevalence in isolates from canines with pyoderma and healthy dogs. *FEMS Microbiol Lett* 312: 169-175.
- Jensen LB, Hammerum AM, Bager F, Aarestrup FM (2002) Streptogramin resistance among *Enterococcus faecium* isolated from production animals in Denmark in 1997. *Microb Drug Resist* 8: 369-374.
- Kadlec K, Schwarz S (2010) Identification of the novel *dftrK*-carrying transposon Tn559 in a porcine methicillin-susceptible *Staphylococcus aureus* ST398 strain. *Antimicrob Agents Chemother* 54: 3475-3477.
- Kadlec K, Schwarz S, Perreten V, Andersson UG, Finn M, Greko C, Moodley A, Kania SA, Frank LA, Bemis DA, Franco A, Iurescia M, Battisti A, Duim B, Wagenaar JA, van Duijkeren E, Weese JS, Fitzgerald JR, Rossano A, Guardabassi L (2010) Molecular analysis of methicillin-resistant *Staphylococcus pseudintermedius* of feline origin from different European countries and North America. *J Antimicrob Chemother* 65: 1826-1828.
- Lautz S, Kanbar T, Alber J, Lämmler C, Weiss R, Prenger-Berninghoff E, Zschöck M (2006) Dissemination of the gene encoding exfoliative toxin of *Staphylococcus intermedius* among strains isolated from dogs during routine microbiological diagnostics. *J Vet Med B Infect Dis Vet Public Health* 53: 434-438.
- Loeffler A, Linek M, Moodley A, Guardabassi L, Sung JM, Winkler M, Weiss R, Lloyd DH (2007) First report of multiresistant, *mecA*-positive *Staphylococcus intermedius* in Europe: 12 cases from a veterinary dermatology referral clinic in Germany. *Vet Dermatol* 18: 412-421.
- Morris DO, Rook KA, Shofer FS, Rankin SC (2006) Screening of *Staphylococcus aureus*, *Staphylococcus intermedius*, and *Staphylococcus schleiferi* isolates obtained from small companion animals for antimicrobial resistance: a retrospective review of 749 isolates (2003 - 04). *Vet Dermatol* 17: 332-337.
- Norström M, Sunde M, Tharaldsen H, Mørk T, Bergsjø B, Kruse H (2009) Antimicrobial resistance in *Staphylococcus pseudintermedius* in the Norwegian dog population. *Microb Drug Resist* 15: 55-59.
- Penna B, Varges R, Medeiros L, Martins GM, Martins RR, Lilenbaum W (2010) Species distribution and antimicrobial susceptibility of staphylococci isolated from canine otitis externa. *Vet Dermatol* 21: 292-296.
- Perreten V, Kadlec K, Schwarz S, Grönlund Andersson U, Finn M, Greko C, Moodley A, Kania SA, Frank LA, Bemis DA, Franco A, Iurescia M, Battisti A, Duim B, Wagenaar JA, van Duijkeren E, Weese JS, Fitzgerald JR, Rossano A, Guardabassi L (2010) Clonal spread of methicillin-resistant *Staphylococcus pseudintermedius*

- in Europe and North America: an international multicentre study. *J Antimicrob Chemother* 65: 1145-1154.
- Perreten V, Vorlet-Fawer L, Slickers P, Ehricht R, Kuhnert P, Frey J (2005) Microarray-based detection of 90 antibiotic resistance genes of gram-positive bacteria. *J Clin Microbiol* 43: 2291-2302.
- Pomba C, Couto N, Moodley A (2010) Treatment of a lower urinary tract infection in a cat caused by a multi-drug methicillin-resistant *Staphylococcus pseudintermedius* and *Enterococcus faecalis*. *J Feline Med Surg* 12: 802-806.
- Poulsen AB, Skov R, Pallesen LV (2003) Detection of methicillin resistance in coagulase-negative staphylococci and in staphylococci directly from simulated blood cultures using the EVIGENE MRSA Detection Kit. *J Antimicrob Chemother* 51: 419-421.
- Ruscher C, Lübke-Becker A, Semmler T, Wleklinski CG, Paasch A, Soba A, Stamm I, Kopp P, Wieler LH, Walther B (2010) Widespread rapid emergence of a distinct methicillin- and multidrug-resistant *Staphylococcus pseudintermedius* (MRSP) genetic lineage in Europe. *Vet Microbiol* 144: 340-346.
- Ruscher C, Lübke-Becker A, Wleklinski CG, Soba A, Wieler LH, Walther B (2008) Prevalence of methicillin-resistant *Staphylococcus pseudintermedius* isolated from clinical samples of companion animals and equidae. *Vet Microbiol* 136: 197-201.
- Ruzauskas M, Couto N, Belas A, Klimiene I, Siugzdiniene R, Pomba C (2013) First report of swine-associated methicillin-resistant *Staphylococcus aureus* ST398 in Lithuania. *Pol J Vet Sci* 16: 125-127.
- Sasaki T, Tsubakishita S, Tanaka Y, Sakusabe A, Ohtsuka M, Hirotaki S, Kawakami T, Fukata T, Hiramatsu K (2010) Multiplex-PCR method for species identification of coagulase-positive staphylococci. *J Clin Microbiol* 66: 765-769.
- Schnellmann C, Gerber V, Rossano A, Jaquier V, Panchaud Y, Doherr MG, Thomann A, Straub R, Perreten V (2006) Presence of new *mecA* and *mph(C)* variants conferring antibiotic resistance in *Staphylococcus* spp. isolated from the skin of horses before and after clinic admission. *J Clin Microbiol* 44: 4444-4454.
- Schwarz S, Kadlec K, Strommenger B (2008) Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* detected in the BfT-GermVet monitoring programme 2004-2006 in Germany. *J Antimicrob Chemother* 61: 282-285.
- Scientific Advisory Group on Antimicrobials of the Committee for Medicinal Products for Veterinary Use (SAGAM) (2009) Reflection paper on the use of third and fourth generation cephalosporins in food producing animals in the European Union: development of resistance and impact on human and animal health. *J Vet Pharmacol Ther* 32: 515-533.
- Seputiene V, Bogdaite A, Ruzauskas M, Suziedeliene E (2012) Antibiotic resistance genes and virulence factors in *Enterococcus faecium* and *Enterococcus faecalis* from diseased farm animals: pigs, cattle and poultry. *Pol J Vet Sci* 15: 431-438.
- Stegmann R, Burnens A, Maranta CA, Perreten V (2010) Human infection associated with methicillin-resistant *Staphylococcus pseudintermedius* ST71. *J Antimicrob Chemother* 65: 2047-2048.
- van Duijkeren E, Catry B, Greko C, Moreno MA, Pomba MC, Pyörälä S, Ruzauskas M, Sanders P, Threlfall EJ, Torren-Edo J, Törneke K (2011) Review on methicillin-resistant *Staphylococcus pseudintermedius*. *J Antimicrob Chemother* 66: 2705-2714.
- van Hoovels L, Vankeerberghen A, Boel A, van Vaerenbergh K, De Beenhouwer H (2006) First case of *Staphylococcus pseudintermedius* infection in a human. *J Clin Microbiol* 44: 4609-4612.
- Weese JS, van Duijkeren E (2010) Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* in veterinary medicine. *Vet Microbiol* 140: 418-429.
- Windahl U, Reimegard E, Holst BS, Egenvall A, Fernstrom L, Fredriksson M, Tronwald-Wigh G, Andersson UG (2012) Carriage of methicillin-resistant *Staphylococcus pseudintermedius* in dogs – a longitudinal study. *BMC Vet Res* 8: 34.